

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☐ ☒ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Trim Galore (version 0.2.7); STAR (2.4.1d); edgeR R package; limma package; sva Bioconductor package; cytoscape app ClueGO (version 2.3.3); ShapIT v2; ADMIXTURE; Plink; R package Matrix eQTL; R package relaimpo; VCFtools v0.1.12b; Selscan
Data analysis	Data analyses were performed using the software packages described above. For identifying genes differentially expressed between population we used linear models. A detailed description of each of the models used is provided in the material and methods.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

RNA-Sequencing data is deposited in the Gene Expression Omnibus with the accession number GSE120502. The 1M SNP genotype data are available at the European Genome-Phenome archive: www.ebi.ac.uk/ega/ (accession numbers EGAS00001000605 and EGAS00001000908).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	In our study we compared the immune response of two populations that have historically resided in different ecologies and maintained different sustenance strategies. The goal was to determine how variation in immune response has been shaped by natural selection. The populations included in this study were the Batwa hunter-gatherers (N = 56) and the Bakiga agriculturalists (N = 47). We separated peripheral blood mononuclear cells (PBMC) and serum from whole blood. We challenged PBMCs with ligands to mimic infection with either a bacteria (lipopolysaccharide) or a virus (Gardiquimod). We then collected genotypes, RNA-sequencing profiles, and sequenced for anti-viral antibodies.
Research sample	Our research samples included whole blood from 103 individuals belonging to two human populations residing in Uganda: the Batwa hunter-gatherer population and the Bakiga agricultural population. Samples were all adults and included both men and women. We chose these populations because they have historically practiced different sustenance strategies and occupied different ecologies but now live in close proximity to one another.
Sampling strategy	Decisions on sample size were based on our past experience with mapping eQTL in the context of immune responses to infection.
Data collection	Blood samples were taken from a total of 103 individuals, 59 HG-Batwa (Hunter-gatherer) and 44 AG-Bakiga (Bantu speaking agriculturalist) individuals. We restricted our sample collection to adult individuals. For the HG-Batwa, we only collected samples from individuals who had lived in the forest and that were born prior to the 1991 formation of Bwindi Impenetrable Forest National Park, a time point known well to the HG-Batwa. PBMCs were collected and processed for both populations simultaneously during the same field expedition to minimize technical variability.
Timing and spatial scale	PBMCs were collected by Dr. Barreiro and Dr. Perry within a two weeks time window back in 2011. Cryopreserved PBMCs were processed in the lab and stimulated over a period of ~2 months.
Data exclusions	Samples were excluded if the data did not meet quality control thresholds.
Reproducibility	This is a population-level analyses and therefore we do not have multiple samples from the same individual. Reproducibility comes from the large number of samples obtained in each of the populations studied, and the statistical model that provide statistical support to the differences in gene expression and immune response detected.
Randomization	To avoid batch effects among different experimental conditions, cDNA libraries were pooled with six libraries per pool in equimolar amounts.
Blinding	N/A
Did the study involve field work?	☒ Yes ☐ No

Field work, collection and transport

Field conditions	Samples were collected from multiple settlements around the Bwindi Impenetrable Forest National Park in Uganda.
Location	Bwindi Impenetrable Forest National Park in Uganda
Access and import/export	All biological samples were sent to the US under an export permit obtained from the Ugandan government.
Disturbance	NA

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Covariates included in our models included sex, genetic ancestry and variation in cellular composition of PBMCs, as measured by flow cytometry.
Recruitment	We restricted our sample collection to adult individuals. For the HG-Batwa, we only collected samples from individuals who had lived in the forest and that were born prior to the 1991 formation of Bwindi Impenetrable Forest National Park, a time point known well to the HG-Batwa.
Ethics oversight	Samples were collected under informed consent (Institutional Review Board protocols 2009-137 from Makerere University, Uganda and 16986A from the University of Chicago). The project was also approved by the Uganda National Counsel for Science and Technology (HS617). This study was approved by the ethics committee of CHU Sainte Justin (project # 2016-1215).

Note that full information on the approval of the study protocol must also be provided in the manuscript.